REMARKS

Reconsideration is respectfully requested of the Office Action of January 27, 2003.

A two-month extension of time, together with the associated fee is filed herewith.

The claims in the case are 1-15 and certain claims have been amended to overcome all the issues relating to 35 U.S.C. § 112. Applicants have made an effort to present more legible representations of the various structures found through out the case.

The rejection of Claims 1-11 under 35 U.S.C. § 103 in view of *Norrgren* and *Chen*, taken with *Wilbur*, is traversed and reconsideration is respectfully requested.

The conditioning of extra corporeal devices by the use of biotin dimers containing "a toxin binding moiety" as means of immobilizing specific ligands to an adsorbent coated with a biotin binding substance is novel and has to applicants' knowledge never been described before. Although biotin dimers containing a third functional group are briefly mentioned in (WO 97/29114, *Wilbur et. al.*), i.e. biotinylation reagents "linked to a reactive moiety that provides a site for reaction with yet another moiety, such as a targeting, diagnostic or therapeutic functional moiety" (page 5, lines 19-20), no reference whatsoever is given to the use of these compounds in any type of applications where these compounds are linked to any type of matrices and no references are given to their applications in affinity chromatography or extra corporeal processes of blood or plasma.

The Office Action states that "Wilbur et al. teach the instant trifunctional linking compounds as useful for adsorbing to a column for extracting various compounds and refers to page 2, lines 1-2, in *Wilbur*. Applicants disagree with the statement in the Office Action. The

referred lines state that "Purification techniques such as affinity chromatography frequently employ biotinylated materials." This is a part of the general description under "Background of the Invention" as to how "avidin and streptavidin renders biotin compound useful for numerous applications" of biotinylated material. *Wilbur* does not state that the biotin compounds described herein would in any way be more suitable or at all suitable for such applications.

On the contrary, Wilbur is focused on the design and synthesis of water-soluble biotin compounds and their clinical applications. The intended therapeutic or diagnostic role of the compounds comprising "biotin dimers containing a third functional group" described in Wilbur are the use of the compounds for polymerizing biotin-binding proteins in vivo by increasing the amount of radioactivity, photoactive moieties, or drugs at a pre-selected site, such as a tumor, by introducing new biotin steps for biotin-binding proteins to bind to. The purpose is to amplify the accumulation of anti-tumor agent to targeted tumor tissue. Hence, these methods of *Wilbur* are completely different from what is taught in the present invention, both with respect to intended use and technical applications. There are no suggestions or any hints to the applicability of these "biotin dimers containing a third functional group" of *Wilbur* to the present invention. More specifically, there are no references to any conditioning of a extra corporeal device or any other means of blood clearance. Applicants note that none of the compounds described in *Wilbur* could be used as "biotin dimers containing a toxin binding moiety" in accordance with the present invention, particularly not since the water-solubility is not an absolute requirement for biotin-compound to be used in the present application.

As noted by *Wilbur*, the biotin/avidin system is widely used for the retrieval or removal of biotinylated species, such as hormone receptors or a variety of biological active compounds. Principally, two different constructs are commonly used. Either the active compound to be removed is biotinylated (with or without a linker serving as a spacer) or a ligand with a certain degree of affinity for the active compound is biotinylated and bound to the immobilized avidin. This linkage can be reversible under certain condition or by using certain types of biotin derivatives.

While affinity chromatography and extra corporeal adsorption have common features, they also differ with respect to a number of features. The present invention is an improvement of these techniques and moreover solve particular problems associated with extra corporeal technologies, such as stability of immobilized proteins, retained physical properties of the device and minimization of undesirable adsorption of endogenous components to the device.

A two-point attachment of biotin derivatives to avidin will enhance the stability of the linkage. One has to bear in mind that by immobilizing the avidin molecule the binding affinity will decrease several orders of magnitudes, and a strengthening of the linkage could therefore be of importance. It is also known that saturation of avidin with biotin or biotin derivatives will enhance the stability of the avidin molecule itself with respect to denaturating conditions.

Moreover, by using the right length of the soluble linker as taught in the present invention the two biotin groups will bind to adjacent binding sites (cross-linking of avidin sub-units) on the same (strept)avidin molecule and thereby avoiding any intermolecular cross-linking of different avidin molecules, and thereby altering the physical properties of the solid support. This is of

vital importance, in particular, in the processing of whole blood, where uncontrolled alterations of the physical properties of the solid support through cross-linking could have a severe effect on the biocompatibility and binding accessibility of such a device.

Still further, by using biotin dimers linked to macromolecules like immunoglobulins all the biotin binding sites can be occupied prior to the exposure to blood and plasma, since an immobilized avidin can only accommodate two molecules of that size due to sterical hindrance. Hence, if macromolecules were biotinylated by the prior art procedure, half of the accessible biotin binding would remain unoccupied and accessible to binding of endogenous biotin and biotin complexes.

These features make the present invention ideal as an technology platform for the making of specific extra corporeal devices carrying various types of "toxin binding moieties" enabling conversion to a variety of different forms, each of them tailor-made for the specific extra corporeal treatment. By applying the methods and reagents taught in the present invention, such a "conditioning step" would be achieved without the addition of any organic solvent, or other chemicals, and could occur by recirculation at ambient temperature, either at an ordinary manufacturing facility or at the hospital site by the use of a blood monitor, prior to treatment.

It may also be noted that in another embodiment of this invention, biotin trimers are utilized to convert a biotin binding device to a (strept) avidin binding device. This can best be achieved by the use of a biotin trimer, since a biotin dimer will, dependent on the length of the linker, either cross-link two adjacent biotin binding sub-units (intra-molecular cross-linking) or cross-link different avidin molecules (inter-molecular cross-linking) as taught in WO 97/29114

and references therein. In either case, no biotin group will be available for the removal or retrieval of biotin binding molecules passing through the device.

The combination of e.g. an avidin coated device with biotin trimers permits the avidin coated device to be used as a platform technology both for the removal of non-toxic targeting agents, and after a single step conditioning procedure as a device for the removal of the toxic material. Various applications of the two forms of an avidin-coated device in multi-step pretargeting are shown in examples 5 and 6. As for the above embodiment, such "conditioning" will occur without organic solvent or other chemicals and would be performed by recirculation at ambient temperature, either at an ordinary manufacturing facility or at the hospital site by the use of a blood monitor, either manually, or pre-programmed, prior to treatment.

This particular use of biotin derivatives has not been foreseen or anticipated, since there are no references to such applications in any of the cited publications. *Chen, et al.*, and *Norrgren, et al.*, describe the use of an extra corporeal device to clear the blood from surplus radioactive biotinylated anti-tumor antibodies and the effect of the method to increase Tumor-to-Normal Tissue Ratio. Although, both discuss the state of the art with respect to various means of improving the efficiency of tumor targeting specifically, they do not discuss conditioning of their device for removal of other toxic material than radiolabelled biotinylated anti-tumor antibodies from the blood circulation. Nor do the references address the issue of extra corporeal removal of any endogenous substance whatsoever or how an avidin coated adsorbent could be converted or used in different contexts other than clearing blood from biotinylated antibodies. It is notable that an avidin coated device can per se only be used to remove either exogenous biotinylated

substances or endogenous biotin. Hence, *Chen* and *Norrgren* would not alone, or in conjunction with WO 97/27114 (*Wilbur*), give any suggestion of the present invention either with respect to the removal of endogenous substances or biotin binding molecules.

In summary, a person skilled in the art, having access to the cited references mentioned above and aiming to solve the problem behind the present invention would not have any incentive to combine any of these references and arrive at the present invention as defined by Claims 1-11.

The above comments apply as well to new Claims 12-15.

To establish a *prima facie* obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure, *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification." *In re Linter,* 458 F.2d 1013, 173 USPQ 560, 562 (CCPA 1972).

Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. *In re Fine,* 837 F2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones,* 958 F2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills,* 916837 F2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

A statement that the modifications of the prior art to meet the claimed invention would have been "'well within the ordinary skill of the art at the time the claimed invention was made'" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* obviousness without some objective reason to combine the teachings of the references. *Ex parte Levengood,* 28 USPQ 2d, 1300 (Bd. Pat. App. & Int. 1993).

For reasons set forth above, applicants submit the Official Action of January 27, 2003, fails to establish *prima facie* obviousness of the claimed invention. Accordigly, applicants respectfully request withdrawal of the rejection.

Favorable action at the Examiner's earliest convenience is respectfully requested.

Respectfully submitted,

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streptavidin that has a therapeutic radionuclide bound to it. In the examples shown (compounds 1-3) the conditioning are the same except for the nature and length of the linker group. The cross-linking reagent employed is 1,3,5-benzene tricarboxylic acid, and the linkers used contain ether functionalities for water solubilization. In compound 3, the linker also contains aspartic acid which provides a free carboxylate to aid in water solubilization and to block the action of biotinidase.

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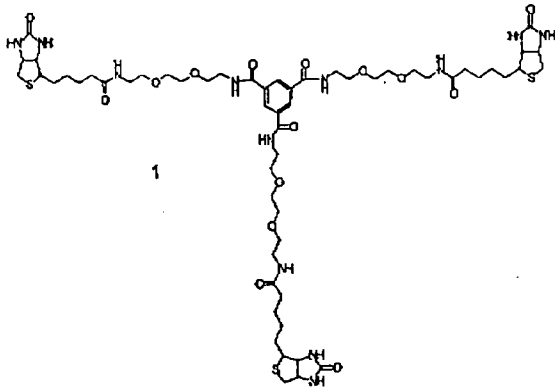
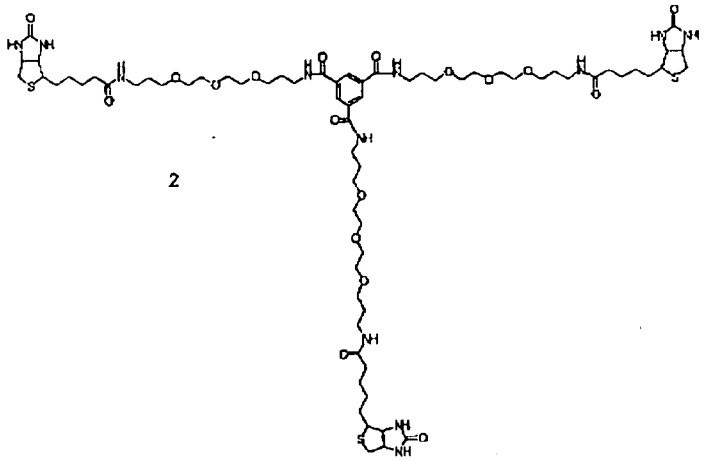
Scheme 1: Examples of biotin dimers that also contain a third biotin for binding radiolabeled streptavidin derivatives

A. Biotin trimer with 31 Å between biotin carboxylates



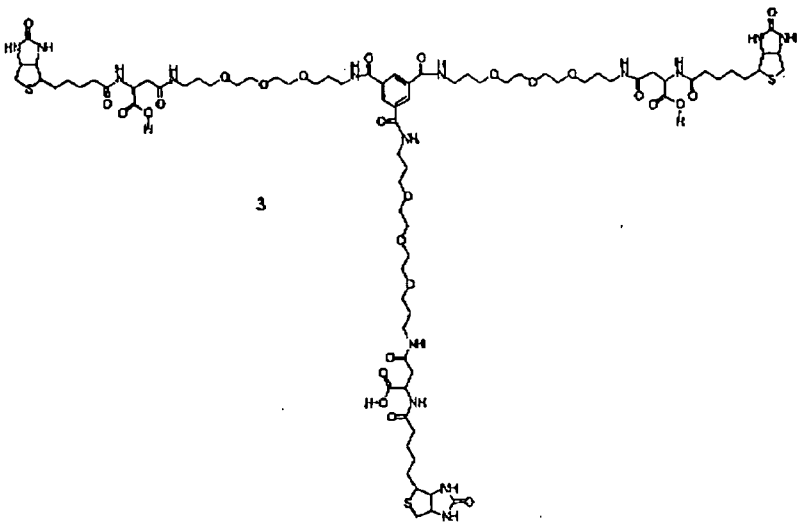
1

B. Biotin trimer with 41 Å between biotin carboxylates



2

C. Biotin trimer with 53 Å between biotin carboxylates



3

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Other toxic material binding dibiotin conditioning reagents involved in the methods according to the present invention can be readily prepared by conjugating a nucleophile containing, or nucleophile reactive, dibiotin

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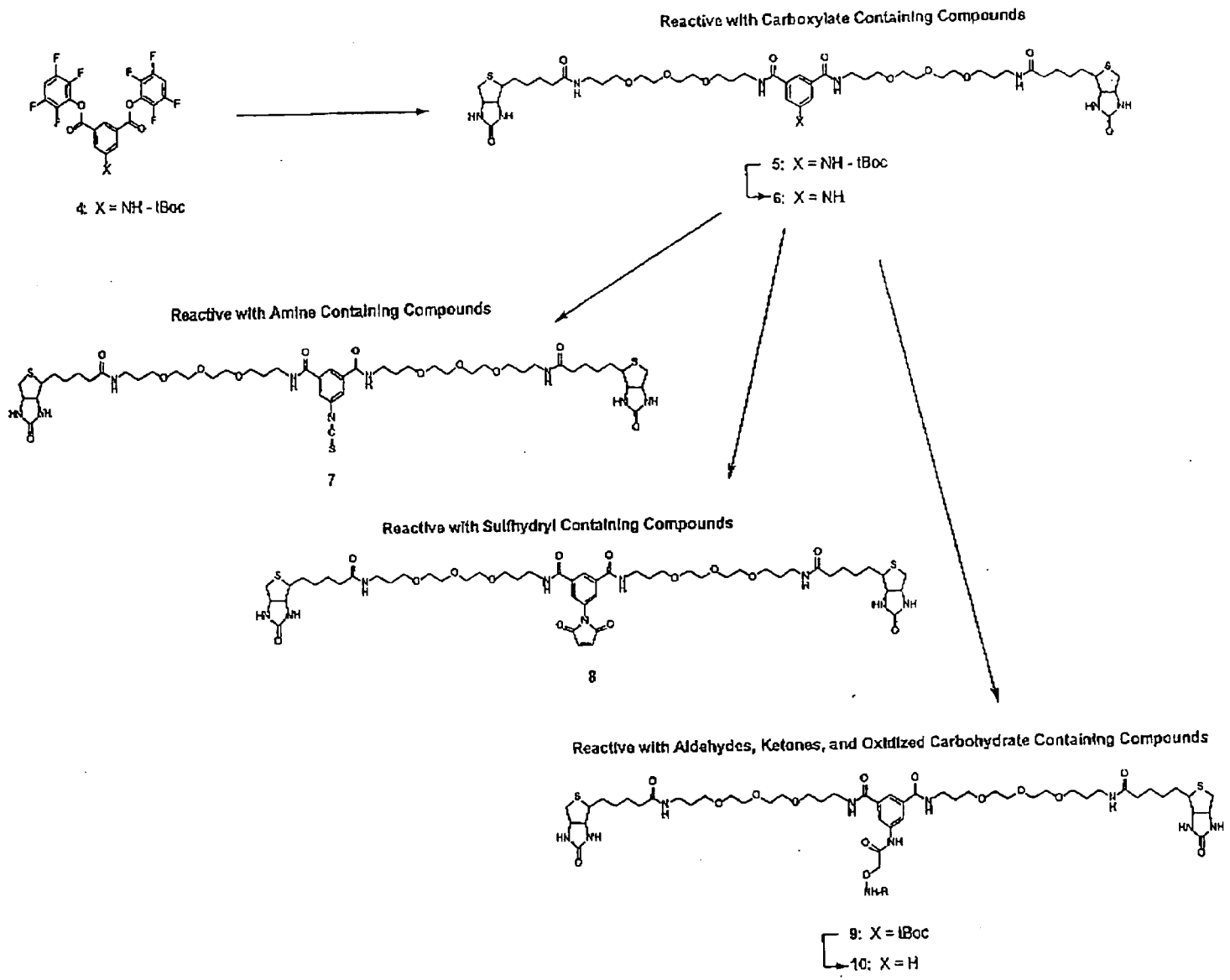
Scheme 2: Synthesis of dibiotin reagents that can be conjugated with other molecules



Examples

The following examples are provided to show methods for obtaining various tpes of compounds disclosed in this patent and their use as a reagent I conditioning the column for toxin removal from whole blood. The examples are provided by way of illustration, and not by way of limitation. Many further examples can be envisioned from the examples shown here.

Example 1

Preparation of a dibiotin compound that can be conjugated with toxin binding molecules

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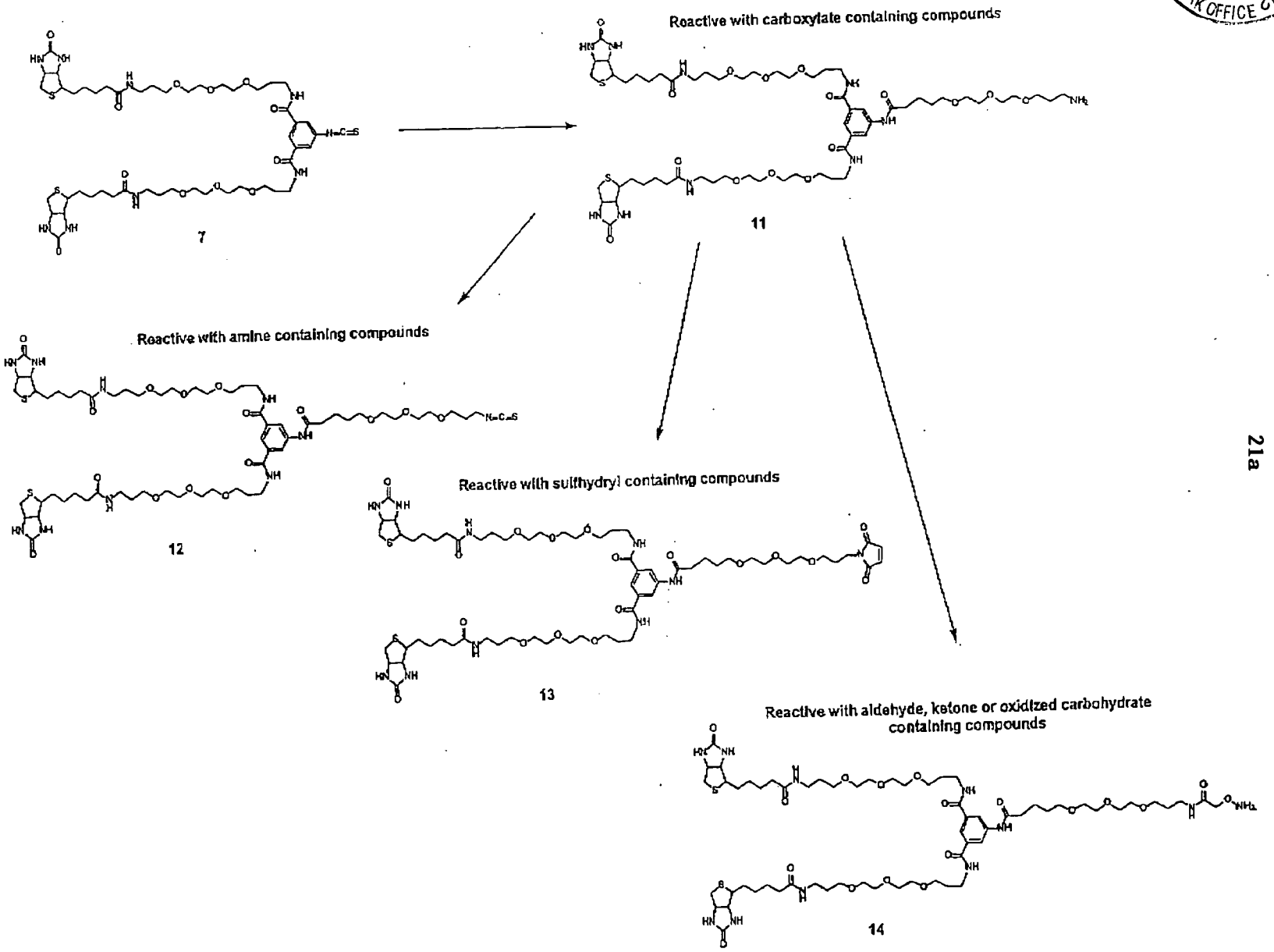
Scheme 3: Synthesis of dibiotin reagents which contain a linker moiety and have functional groups which permit conjugation with other molecules



(0.52 mL, 3.70 mmol), DMF (4.0 mL) and water (10 mL) at room temperature. A 60 mL quantity of water was added after the reaction mixture was stirred at room temperature for 30 min, the white precipitate was filtered, washed with water, dried in vacuo to give the crude product. The crude product was purified by silica gel column chromatography (40 g) eluting with 10% EtOAc/hexane to give a colorless solid. Yield 0.213 g (30%). mp 159.7-161.8°C dec. 1H NMR (CDCl3):
1.55 (s, 9H), 6.83 (s, 1H), 7.08 (m, 1H), 8.54 (d, J = 1.5 Hz, 2H), 8.67 (t, J = 1.5 Hz, 1H). HRMS calcd for $C_{25}H_{15}F_8NNaO_6$ (M + Na)+: 600.0669. Found: 600.0674. HPLC: tR = 16.1 min.

Step 3: Preparation of N-(13-amino-4,7,10-trioxa-tridecanyl)biotinamide.

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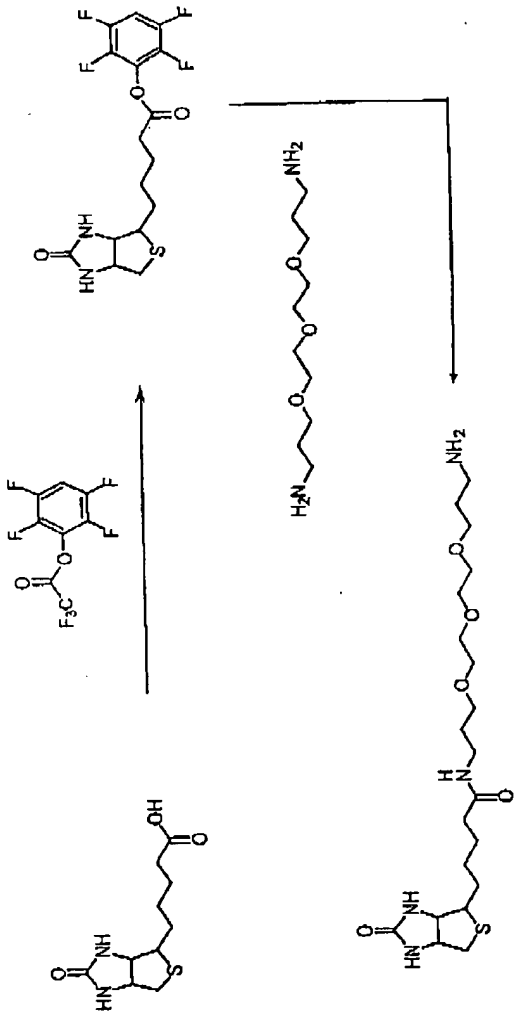
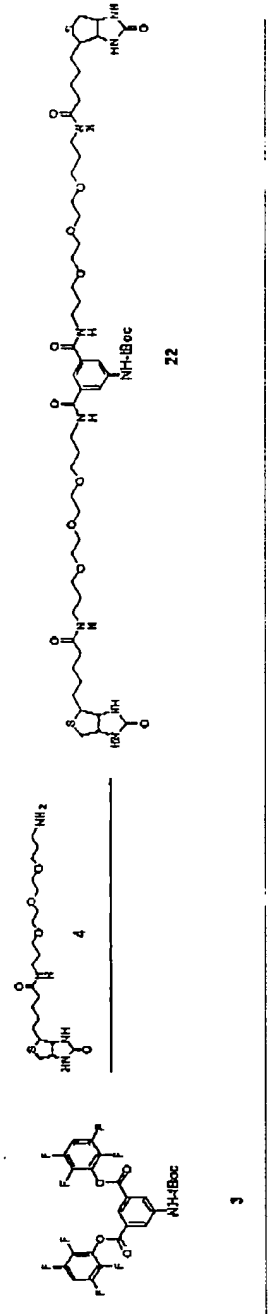


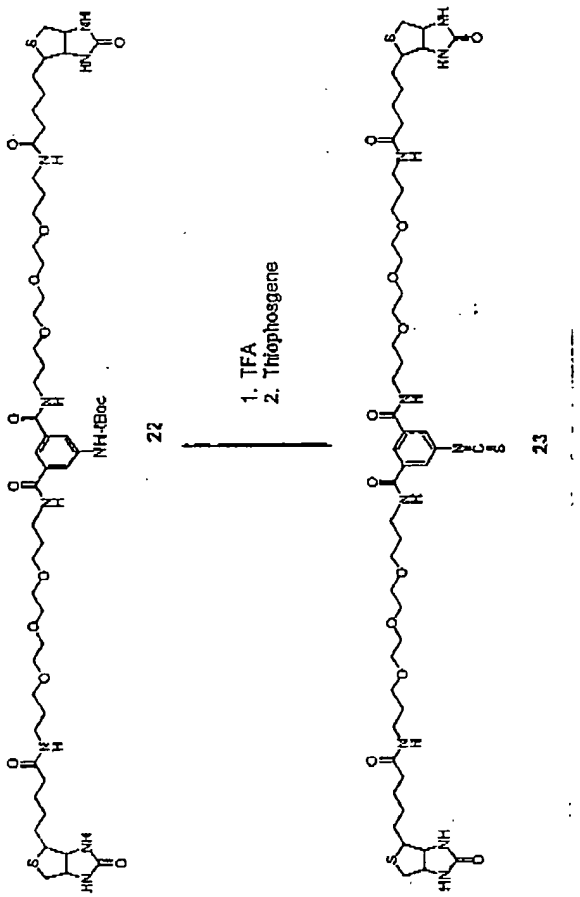
Biotin (10 g, 40.9 mmol) was dissolved in 200 mL warm (70°C) DMF under argon atmosphere. The solution was allowed to cool to ambient temperature, 10 mL (82 mmol) triethylamine was added, followed by the addition of 16 g (61 mmol) 2,3,5,6-tetrafluorophenyl trifluoroacetate. The reaction was stirred at room temperature for 30 min

Step 4: 1-N-tert-Boc-3,5-Bis(13´-(biotinamidyl)-4´,7´,10´-trioxatridecanamidyl)-aminoisophthalate.


NH-tBoc
22
4
3
NH-tBoc

Biotin-trioxadiamine, 4 (100 mg, 0.22 mmol) in anhydrous DMF was added dropwise to a solution of 3 (65 mg, 0.11 mmol) and triethylamine (47 µL, 0.33 mmol) in anhydrous DMF at rt (room temperature). The reaction mixture was stirred at rt for 2 h, and then the solution was evaporated to dryness under vacuum. The residue was purified by silica gel column (40 g) eluting with 20% MeOH/EtOAc to yield 73 mg (58%) of a colorless solid, mp 209-211°C dec. 1H NMR (CD_3OD, 200 MHz): δ1.43 (t, 3H), 1.54 (s, 9 H), 1.69 (m, 6H), 1.88 (m, 3H), 2.19 (m, 4H), 2.69 (d, 4H), 2.92 (m, 2H), 4.30 (m, 2H), 4.48 (m, 2H), 7.83 (m, 1H), 8.00 (m, 2H); mass calcd for $C_{53}H_{88}N_9O_{14}S_2$ (M + H))+: 1139. Found: 1139. mass calcd for $C_{53}H_{87}N_9O_{14}S_2Na$ (M + Na)+: 1161. Found: 1161. HPLC 11.8 min.

Step 5: 1-Isothiocyanato-3,5-Bis(13´-(biotinamidyl)-4´,7´,10´-trioxatridecandyl)-aminoiso-phthalate


NH-tBoc
22
1. TFA
2. Thiophosgene
NCS
23

Replacement Page

A 120 mg quantity of 22 (0.11 mmol) was dissolved in neat TFA (1 mL) and stirred at rt for 10 min. Following this, excess TFA was removed under vacuum. The residue was dissolved in 2 mL of methanol and treated with 0.2 mL of triethylamine. The volatile materials were removed under vacuum, then water (3 mL), chloroform (3 mL) and thiophosgene (42 µL, 0.55 mmol) were added respectively. The mixture was stirred at rt for 1 h. Following this, excess thiophosgene and chloroform were evaporated in fume hood under a stream of argon. The remaining aqueous phase was evaporated to dryness under vacuum to afford 77 mg (68%) of 23 as a light yellow tacky solid. 1H NMR (DMSO-d6, 200 MHz): δ 1.24-1.35 (m, 6H), 1.43-1.67 (m, 14H), 1.77 (t, J = 6.6 Hz, 6H), 2.05 (t, J = 7.1 Hz, 6H), 2.58 (d, J = 12.5 Hz, 2H), 2.82 (dd, J = 4.8, 12.5 Hz, 2H), 3.07 (m, 8H), 3.28-3.57 (m, 18H), 4.13 (dd, J = 4.6, 7.7 Hz, 2H), 4.31 (dd, J = 4.6, 7.7 Hz, 2H), 7.80 (t, J = 5.0 Hz, 2H), 7.98 (s, 2H), 8.34 (s, 1H), 8.77 (t, J = 5.1 Hz, 2H) mass calcd for $C_{49}H_{78}N_9O_{12}S_3Na$ (M + Na)+: 1103. Found: 1103. HPLC 11.8 min.

Conjugation of a dibiotin compound with a toxin binding molecule